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(54) Title: 18615 AND 48003, NOVEL HUMAN ION CHANNELS AND USES THEREFOR

(57) Abstract: The invention provides isolated nucleic acid molecules, designated VR-3 and VR-5 nucleic acid molecules, which are novel molecules which are members of the ion channel, e.g., calcium channel and/or vanilloid receptor, family. The invention also provides antisense nucleic acid molecules, recombinant expression vectors containing VR-3 or VR-5 nucleic acid molecules, host cells into which the expression vectors have been introduced, and nonhuman transgenic animals in which a VR-3 or VR-5 gene has been introduced or disrupted. The invention still further provides isolated VR-3 or VR-5 proteins, fusion proteins, antigenic peptides, and anti-VR-3 or anti-VR-5 antibodies. Diagnosis methods utilizing compositions of the invention are also provided.

18615 AND 48003, NOVEL HUMAN ION CHANNELS AND USES THEREFOR**Related Applications**

- The present application claims priority to prior filed U.S. Patent Application
- 5 Serial No. 09/525,420 entitled "18615 and 48003, Novel Human Calcium Channel/Vanilloid Receptors and Uses Therefor", filed March 15, 2000. The content of the above-referenced patent application is incorporated herein by this reference in its entirety.

10 Background of the Invention

- Calcium is the most abundant cation in the human body and plays a critical role in many physiologic processes. It is an essential component of bone as well as a well-known first and second messenger in signal transduction. Intracellular calcium regulates cell functions such as membrane excitability, release of neurotransmitters, muscle
- 15 contraction, hormonal secretion, glycogen metabolism, and cell division. Extracellular calcium ensures the steady supply of intracellular calcium and also has other important functions, for example it plays a role in cell-to-cell adhesion and blood clotting. Intestinal absorption is the only way that calcium may enter the body. The human dietary intake of calcium is normally less than 1000 mg per day, of which only 30% is
- 20 absorbed. This absorption occurs throughout the small intestine both through active transport (vitamin D-dependent) and by passive diffusion. Calcium is excreted primarily through the kidneys, although 95% or more is reabsorbed (resorption). Renal tubular resorption of filtered calcium is mainly regulated by the parathyroid hormone.

- It is essential that homeostasis of calcium levels is precisely controlled. About
- 25 0.1% of the total body calcium is contained in the blood and extracellular compartment (van Os (1987) *Biochim. Biophys. Acta*, 906:195-222). This calcium pool is maintained in equilibrium with the large calcium stores controlled by the bone, kidneys, and intestine. It is in these tissues where the bulk of calcium flux across membranes occurs in response to homeostatic cues.

- 30 Perturbations in calcium homeostasis are features of many pathological states (Birge and Avioli, *Clinical Disorders of Membrane Transport Processes*, Andreoli *et al.* Eds., Plenum Press, New York, 1987, pp. 121-140). For example, in osteoporosis,

increased resorption of bone elevates serum calcium levels, which in turn depresses the activity of parathyroid hormone. This has the effect of decreasing renal tubular resorption of calcium, which causes net urinary loss of total calcium. Other disorders associated with aberrant calcium absorption and homeostasis include intrinsic bowel
5 disease, hepatobiliary disease, renal disease, idiopathic hypercalciuric syndromes, hypoparathyroidism, hyperthyroidism and central nervous system (CNS) disorders such as those involving neurotransmitter release (*e.g.*, Alzheimer's and Parkinson's disease).

The TRP channel family is one of the best characterized members of the capacitativative calcium channel group. These channels include transient receptor potential
10 protein and homologues thereof (to date, seven homologs and splice variants have been identified in a variety of organisms), the vanilloid receptors (also known as the capsaicin receptors), stretch-inhibitable non-selective cation channel (SIC), olfactory, mechanosensitive channel, insulin-like growth factor I-regulated calcium channel, and vitamin D-responsive apical, epithelial calcium channel (ECaC), melastatin, and the
15 polycystic kidney disease protein family (see, *e.g.*, Montell and Rubin (1989) *Neuron* 2:1313-1323; Caterina *et al.* (1997) *Nature* 389: 816-824; Suzuki *et al.* (1999) *J. Biol. Chem.* 274: 6330-6335; Kiselyov *et al.* (1998) *Nature* 396: 478-482; Hoenderop *et al.* (1999) *J. Biol. Chem.* 274: 8375-8378; and Chen *et al.* (1999) *Nature* 401(6751): 383-6). Each of these molecules is 700 or more amino acids in length (TRP and TRP homologs
20 have 1300 or more amino acid residues), and shares certain conserved structural features. Predominant among these structural features are six transmembrane domains, with an additional hydrophobic loop present between the fifth and sixth transmembrane domains. It is believed that this loop is integral to the activity of the pore of the channel formed upon membrane insertion (Hardie and Minke (1993) *Trends Neurosci* 16: 371-
25 376). TRP channel proteins also include one or more ankyrin domains and frequently display a proline-rich region at the N-terminus. Although found in disparate tissues and organisms, members of the TRP channel protein family all serve to transduce signals by means of calcium entry into cells, particularly pain signals (see, *e.g.*, McClesky and Gold (1999) *Annu. Rev. Physiol.* 61: 835-856), light signals (Hardie and Minke, *supra*),
30 or olfactory signals (Colbert *et al.* (1997) *J. Neurosci* 17(21): 8259-8269). Thus, this family of molecules may play important roles in sensory signal transduction in general.

Melastatin, a gene with homology to members of the TRP channel family, has been shown to be involved in cancer (*e.g.*, melanoma). Expression of melastatin is inversely correlated with melanoma aggressiveness such that melastatin expression was found to be downregulated in metastatic melanomas. Melastatin mRNA expression is also variably down-regulated in melanomas of intermediate thickness. These findings suggest that melastatin has a role as a suppressor of melanoma metastasis or an inhibitor of melanoma tumor progress and may be utilized as a marker for metastasis in patients with localized malignant melanoma (Duncan, *et al.* (1998) *Cancer Research* 58(7):1515-1520; Deeds, *et al.* (2000) *Hum Pathology* 31(11):1346-56; Enklaar *et al.* (2000) *Genomics* 67(2):179-87; Duncan *et al.* (2001) *J Clin Oncol* 19(2):568-576).

Vanilloid receptors (VRs) are non-selective cation channels that are structurally related to members of the TRP family of ion channels. These receptors have been proposed to mediate the entry of extracellular calcium into cells in response to the depletion of intracellular calcium stores. VRs are expressed in nociceptive neurons, as well as other cells types, and are activated by a variety of stimuli including noxious heat and protons. Capsaicin, which is a well-known agonist of VRs, induces pain behavior in humans and rodents. VR-1, a vanilloid receptor, was identified in rat sensory ganglia and is involved in pain signaling and nociception (Caterina M. J. *et al.*, (1997) *Nature* 389:816-824).

Summary of the Invention

The present invention is based, at least in part, on the discovery of novel molecules which are members of the ion channel, *e.g.*, calcium channel and/or vanilloid receptor, family, referred to herein as "Vanilloid Receptor 3", "Vanilloid Receptor 5", "VR-3", or "VR-5" nucleic acid and protein molecules. The VR-3 or VR-5 molecules of the present invention are useful as targets for developing modulating agents to regulate a variety of cellular processes, including cellular processes involved in the development and regulation of pain, as well as homeostasis of calcium levels. Furthermore, based on the discovery that the VR-3 or VR-5 molecules of the present invention are differentially expressed in tumors, *e.g.*, lung, ovarian, breast, prostate, colon, and Wilms tumors, compared to normal tissues, *e.g.*, normal lung, ovarian, breast, prostate, colon, and kidney tissue, respectively, these molecules may useful in the

diagnosis and treatment of cellular growth and proliferation disorders, *e.g.*, cancer, including, but not limited to, lung cancer, ovarian cancer, breast cancer, prostate cancer, colon cancer, or kidney cancer. Accordingly, in one aspect, this invention provides isolated nucleic acid molecules encoding VR-3 or VR-5 proteins or biologically active portions thereof, as well as nucleic acid fragments suitable as primers or hybridization probes for the detection of VR-3-encoding or VR-5-encoding nucleic acids.

In one embodiment, a VR-3 or VR-5 nucleic acid molecule of the invention is at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 83%, 85%, 86%, 90%, 91%, 92%, 95%, 96%, 97%, 98%, 99%, 99.1%, 99.5% or more identical to the nucleotide sequence (*e.g.*, to the entire length of the nucleotide sequence) shown in SEQ ID NO:1, 3, 4, or 6 or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number PTA-2013, or a complement thereof.

In a preferred embodiment, the isolated nucleic acid molecule includes the nucleotide sequence shown SEQ ID NO:1, 3, 4, or 6, or a complement thereof. In another embodiment, the nucleic acid molecule includes SEQ ID NO:3 and nucleotides 1-277 of SEQ ID NO:1. In another embodiment, the nucleic acid molecule includes SEQ ID NO:3 and nucleotides 2456-3026 of SEQ ID NO:1. In another embodiment, the nucleic acid molecule includes SEQ ID NO:6 and nucleotides 1-83 of SEQ ID NO:4. In another embodiment, the nucleic acid molecule includes SEQ ID NO:6 and nucleotides 2700-3245 of SEQ ID NO:4. In another preferred embodiment, the nucleic acid molecule consists of the nucleotide sequence shown in SEQ ID NO:1, 3, 4, or 6. In another preferred embodiment, the nucleic acid molecule includes a fragment of at least 50, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000, 2100, 2200, 2242, 2300, 2400, 2500, 2600, 2700, 2800, 2900, 3000, 3100, 3200, or more nucleotides (*e.g.*, contiguous nucleotides) of the nucleotide sequence of SEQ ID NO:1, 3, 4, or 6, or a complement thereof.

In another embodiment, a VR-3 or VR-5 nucleic acid molecule includes a nucleotide sequence encoding a protein having an amino acid sequence sufficiently identical to the amino acid sequence of SEQ ID NO:2 or 5 or an amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number PTA-2013. In a preferred embodiment, a VR-3 or VR-5 nucleic acid molecule includes a nucleotide sequence encoding a protein having an amino acid sequence at least 50%,

55%, 60%, 65%, 70%, 75%, 80%, 85%, 86%, 90%, 91%, 95%, 96%, 97%, 98%, 99%, 99.1%, 99.5%, or more identical to the entire length of the amino acid sequence of SEQ ID NO:2 or 5, or the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number PTA-2013.

5 In another preferred embodiment, an isolated nucleic acid molecule encodes the amino acid sequence of human VR-3 or VR-5. In yet another preferred embodiment, the nucleic acid molecule includes a nucleotide sequence encoding a protein having the amino acid sequence of SEQ ID NO:2 or 5, or the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number PTA-2013.

10 In another preferred embodiment, the nucleic acid molecule is at least 362 nucleotides in length. In a further preferred embodiment, the nucleic acid molecule is at least 362 nucleotides in length and encodes a protein having a VR-3 activity (as described herein). In yet another preferred embodiment, the nucleic acid molecule is at least 519 nucleotides in length. In a further preferred embodiment, the nucleic acid
15 molecule is at least 519 nucleotides in length and encodes a protein having a VR-5 activity (as described herein).

Another embodiment of the invention features nucleic acid molecules, preferably VR-3 or VR-5 nucleic acid molecules, which specifically detect VR-3 or VR-5 nucleic acid molecules relative to nucleic acid molecules encoding non-VR-3 or non-VR-5
20 proteins. For example, in one embodiment, such a nucleic acid molecule is at least 100-500, 500-1000, 1000-1500, 1500-2000, 2000-2500, 2500-3000, 3000-3200, or more nucleotides in length and hybridizes under stringent conditions to a nucleic acid molecule comprising the nucleotide sequence shown in SEQ ID NO:1 or 4, the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as
25 Accession Number PTA-2013, or a complement thereof.

In preferred embodiments, the nucleic acid molecules are at least 15 (*e.g.*, contiguous) nucleotides in length and hybridize under stringent conditions to nucleotides 1-65 or 2986-3026 of SEQ ID NO:1. In other preferred embodiments, the nucleic acid molecules comprise nucleotides 1-65 or 2986-3026 of SEQ ID NO:1. In other preferred
30 embodiments, the nucleic acid molecules consist of nucleotides 1-65 or 2986-3026 of SEQ ID NO:1.

In preferred embodiments, the nucleic acid molecules are at least 15 (*e.g.*, contiguous) nucleotides in length and hybridize under stringent conditions to nucleotides 1-31 of SEQ ID NO:4. In other preferred embodiments, the nucleic acid molecules comprise nucleotides 1-31 of SEQ ID NO:4. In other preferred embodiments, the
5 nucleic acid molecules consist of nucleotides 1-31 of SEQ ID NO:4.

In other preferred embodiments, the nucleic acid molecule encodes a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2 or 5, or an amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number PTA-2013, wherein the nucleic acid
10 molecule hybridizes to a nucleic acid molecule comprising SEQ ID NO:1, 3, 4 or 6 under stringent conditions.

Another embodiment of the invention provides an isolated nucleic acid molecule which is antisense to a VR-3 or VR-5 nucleic acid molecule, *e.g.*, the coding strand of a VR-3 or VR-5 nucleic acid molecule.

15 Another aspect of the invention provides a vector comprising a VR-3 or VR-5 nucleic acid molecule. In certain embodiments, the vector is a recombinant expression vector. In another embodiment, the invention provides a host cell containing a vector of the invention. In yet another embodiment, the invention provides a host cell containing a nucleic acid molecule of the invention. The invention also provides a method for
20 producing a protein, preferably a VR-3 or VR-5 protein family member, by culturing a host cell in a suitable medium, *e.g.*, a mammalian host cell such as a non-human mammalian cell, of the invention containing a recombinant expression vector, such that the protein is produced.

Another aspect of this invention features isolated or recombinant VR-3 proteins
25 and polypeptides. In preferred embodiments, the isolated VR-3 protein family member includes at least one or more of the following domains: an ankyrin repeat domain, a pore domain, and/or a transmembrane domain.

Another aspect of this invention features isolated or recombinant VR-5 proteins and polypeptides. In preferred embodiments, the isolated VR-5 protein family member
30 includes at least one or more of the following domains: an ankyrin repeat domain, a pore domain, a transmembrane domains, and/or an ion transport protein domain.

In a preferred embodiment, the VR-3 protein family member has an amino acid sequence at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 86%, 90%, 91%, 95%, 96%, 97%, 98%, 99%, 99.1%, 99.5%, or more identical to the amino acid sequence of SEQ ID NO:2, and includes at least one or more of the following domains:

- 5 an ankyrin repeat domain, a pore domain, and/or a transmembrane domain.

In another preferred embodiment, the VR-5 protein family member has an amino acid sequence at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 86%, 90%, 91%, 95%, 96%, 97%, 98%, 99%, 99.1%, 99.5%, or more identical to the amino acid sequence of SEQ ID NO:5, or the amino acid sequence encoded by the DNA insert of
10 the plasmid deposited with ATCC as Accession Number PTA-2013, and includes at least one or more of the following domains: an ankyrin repeat domain, a pore domain, a transmembrane domains, and/or an ion transport protein domain.

In another preferred embodiment, the VR-3 protein family member plays a role in calcium homeostasis, pain signaling, and/or cellular growth and/or proliferation and
15 includes at least one or more of the following domains: an ankyrin repeat domain, a pore domain, and/or a transmembrane domain.

In another preferred embodiment, the VR-5 protein family member plays a role in calcium homeostasis, pain signaling, and/or cellular growth and/or proliferation and includes at least one or more of the following domains: an ankyrin repeat domain, a pore
20 domain, a transmembrane domains, and/or an ion transport protein domains.

In yet another preferred embodiment, the VR-3 protein family member is encoded by a nucleic acid molecule having a nucleotide sequence which hybridizes under stringent hybridization conditions to a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1 or 3, and includes at least one or more of the
25 following domains: an ankyrin repeat domain, a pore domain, and/or a transmembrane domain.

In yet another preferred embodiment, the VR-5 protein family member is encoded by a nucleic acid molecule having a nucleotide sequence which hybridizes under stringent hybridization conditions to a nucleic acid molecule comprising the
30 nucleotide sequence of SEQ ID NO:4 or 6, and includes at least one or more of the following domains: an ankyrin repeat domain, a pore domain, a transmembrane domains, and/or an ion transport protein domain.

In another embodiment, the invention features fragments of the protein having the amino acid sequence of SEQ ID NO:2 or 5, wherein the fragment comprises at least 15, 20, 30, 40, 50, 60, 70, 80, 90, or 100 amino acids (*e.g.*, contiguous amino acids) of the amino acid sequence of SEQ ID NO:2 or 5, or an amino acid sequence encoded by the DNA insert of the plasmid deposited with the ATCC as Accession Number PTA-2013. In another embodiment, the protein, preferably a VR-3 or VR-5 protein, has the amino acid sequence of SEQ ID NO:2 or 5.

In another embodiment, the invention features an isolated VR-3 or VR-5 protein family member which is encoded by a nucleic acid molecule consisting of a nucleotide sequence at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 82.6%, 85%, 86%, 90%, 95%, 96%, 97%, 98%, 99%, 99.1% or more identical to a nucleotide sequence of SEQ ID NO:1, 3, 4 or 6, or a complement thereof. This invention further features an isolated protein, preferably a VR-3 or VR-5 protein, which is encoded by a nucleic acid molecule consisting of a nucleotide sequence which hybridizes under stringent hybridization conditions to a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, 3, 4 or 6, or a complement thereof.

The proteins of the present invention or portions thereof, *e.g.*, biologically active portions thereof, can be operatively linked to a non-VR-3 or a non-VR-5 polypeptide (*e.g.*, heterologous amino acid sequences) to form fusion proteins. The invention further features antibodies, such as monoclonal or polyclonal antibodies, that specifically bind proteins of the invention, preferably VR-3 or VR-5 proteins. In addition, the VR-3 or VR-5 proteins or biologically active portions thereof can be incorporated into pharmaceutical compositions, which optionally include pharmaceutically acceptable carriers.

In another aspect, the present invention provides a method for detecting the presence of a VR-3 or VR-5 nucleic acid molecule, protein or polypeptide in a biological sample by contacting the biological sample with an agent capable of detecting a VR-3 or VR-5 nucleic acid molecule, protein or polypeptide such that the presence of a VR-3 or VR-5 nucleic acid molecule, protein or polypeptide is detected in the biological sample.

In another aspect, the present invention provides a method for detecting the presence of VR-3 or VR-5 activity in a biological sample by contacting the biological sample with an agent capable of detecting an indicator of VR-3 or VR-5 activity such that the presence of VR-3 or VR-5 activity is detected in the biological sample.

5 In another aspect, the invention provides a method for modulating VR-3 or VR-5 activity comprising contacting a cell capable of expressing VR-3 or VR-5 with an agent that modulates VR-3 or VR-5 activity such that VR-3 or VR-5 activity in the cell is modulated. In one embodiment, the agent inhibits VR-3 or VR-5 activity. In another embodiment, the agent stimulates VR-3 or VR-5 activity. In one embodiment, the agent
10 is an antibody that specifically binds to a VR-3 or VR-5 protein. In another embodiment, the agent modulates expression of VR-3 or VR-5 by modulating transcription of a VR-3 or VR-5 gene or translation of a VR-3 or VR-5 mRNA. In yet another embodiment, the agent is a nucleic acid molecule having a nucleotide sequence that is antisense to the coding strand of a VR-3 or VR-5 mRNA or a VR-3 or VR-5
15 gene.

In one embodiment, the methods of the present invention are used to treat a subject having a disorder characterized by aberrant or unwanted VR-3 or VR-5 protein or nucleic acid expression or activity by administering an agent which is a VR-3 or VR-5 modulator to the subject. In one embodiment, the VR-3 or VR-5 modulator is a VR-3
20 or VR-5 protein. In another embodiment the VR-3 or VR-5 modulator is a VR-3 or VR-5 nucleic acid molecule. In yet another embodiment, the VR-3 or VR-5 modulator is an antibody, ribozyme, peptide, peptidomimetic, antisense oligonucleotide, or other small molecule. In a preferred embodiment, the disorder characterized by aberrant or unwanted VR-3 or VR-5 protein or nucleic acid expression is a calcium homeostasis
25 related disorder. In another preferred embodiment, the disorder characterized by aberrant or unwanted VR-3 or VR-5 protein or nucleic acid expression is cancer, *e.g.*, lung cancer, ovarian cancer, breast cancer, prostate cancer, colon cancer, or Wilms tumors. In yet another preferred embodiment, the disorder characterized by aberrant or unwanted VR-3 or VR-5 protein or nucleic acid expression is pain or a pain disorder.

30 The present invention also provides diagnostic assays for identifying the presence or absence of a genetic alteration characterized by at least one of (i) aberrant modification or mutation of a gene encoding a VR-3 or VR-5 protein; (ii) mis-regulation

of the VR-3 or VR-5 gene; and (iii) aberrant post-translational modification of a VR-3 or VR-5 protein, wherein a wild-type form of the gene encodes a protein with a VR-3 or VR-5 activity.

In another aspect the invention provides methods for identifying a compound
5 that binds to or modulates the activity of a VR-3 or VR-5 protein, by providing an indicator composition comprising a VR-3 or VR-5 protein having VR-3 or VR-5 activity, contacting the indicator composition with a test compound, and determining the effect of the test compound on VR-3 or VR-5 activity in the indicator composition to identify a compound that modulates the activity of a VR-3 or VR-5 protein.

10

Other features and advantages of the invention will be apparent from the following detailed description and claims.

Brief Description of the Drawings

15 *Figure 1* depicts the cDNA sequence and predicted amino acid sequence of the human VR-3. The nucleotide sequence corresponds to nucleic acids 1 to 3026 of SEQ ID NO:1. The amino acid sequence corresponds to amino acids 1 to 725 of SEQ ID NO:2. The coding region of the human VR-3 is shown in SEQ ID NO:3.

Figure 2 depicts the cDNA sequence and predicted amino acid sequence of the
20 human VR-5. The nucleotide sequence corresponds to nucleic acids 1 to 3245 of SEQ ID NO:4. The amino acid sequence corresponds to amino acids 1 to 871 of SEQ ID NO:5. The coding region of the human VR-5 is shown in SEQ ID NO:6.

Figure 3 depicts an alignment of the amino acid sequence of human VR-3 with the rat calcium transporter (GenBank Accession No. AF160798) and the rabbit epithelial
25 calcium channel (GenBank Accession No. AJ133128) using the CLUSTALW (1.74) multiple sequence alignment program.

Figure 4 depicts an alignment of the amino acid sequence of human VR-5 with the amino acid sequence of the *Mus musculus* ion channel (GenBank Accession No. AB021875) using the GAP program in the GCG software package (Blosum 62 matrix)
30 and a gap weight of 12 and a length weight of 4.

Figure 5 depicts an alignment of the nucleotide sequence of human VR-5 with the nucleotide sequence of the *Mus musculus* ion channel (GenBank Accession Number AB021875) using the CLUSTALW (1.74) multiple sequence alignment program.

5 *Figure 6* depicts a structural, hydrophobicity, and antigenicity analysis of the human VR-3 protein.

Figure 7 depicts a structural, hydrophobicity, and antigenicity analysis of the human VR-5 protein.

10 *Figure 8* depicts the results of a search which was performed against the HMM database using the amino acid sequence of human VR-3. This search resulted in the identification of three "ankyrin repeat" domains domain in the human VR-3 protein.

15 *Figure 9* depicts the results of a search which was performed against the HMM database using the amino acid sequence of human VR-5. This search resulted in the identification of three "ankyrin repeat" domains and one "ion transport protein" domain in the human VR-5 protein.

Figure 10 depicts the results of a search performed against the HMM database using the amino acid sequence of human VR-3. This search resulted in the local alignment of the human VR-3 protein with the rat VR-1 protein, the protein olfactory channel/VR, and the transmembrane calcium receptor/ion transport protein.

20 *Figure 11* depicts the results of a search performed against the HMM database using the amino acid sequence of human VR-5. This search resulted in the local alignment of the human VR-5 protein with the rat VR-1 protein, the protein olfactory channel/VR, and the transmembrane calcium receptor/ion transport protein.

25 **Detailed Description of the Invention**

The present invention is based, at least in part, on the discovery of novel molecules which are members of the ion channel, *e.g.*, calcium channel and/or vanilloid receptor, family. Described herein is the isolation of two human ion channels, *e.g.*, calcium channel/vanilloid receptors, referred to herein as "Vanilloid Receptor-3" or
30 "VR-3" or "VR-5" and as "Vanilloid Receptor 5" or "VR-5."

The VR-3 and VR-5 sequences of the present invention are similar to that of rat VR-1. VR-1 is a vanilloid gated, non-selective cation channel which resembles members of the transient receptor potential (TRP) ion channel family (described in Montell *et al.* (1989) *Neuron* 2:1313-1323) that mediate the influx of extracellular calcium in response to depletion of intracellular calcium stores. Hydrophilicity analysis has indicated that rat VR-1 contains six transmembrane domains (predicted to be mostly α -helices). The amino terminal hydrophilic segment contains three ankyrin repeat domains. The rat VR-1 was identified in rat sensory ganglia (Caterina M. J. *et al.*, (1997) *Nature* 389:816-824). It has been shown that VR-1 knockout mice are impaired in their detection of painful heat, exhibit no vanilloid-evoked pain behavior, and show little thermal hypersensitivity after inflammation (Szallasi and Blumberg (1999) *Pharmacol. Rev.* 51:159-211; Tominaga, *et al.* (1998) *Neuron* 21:531; Caterina *et al.* (2000) *Science* 288:306). Based on homology to VR-1 and the discovery that VR-3 and VR-5 are expressed in brain (*e.g.*, cortex and hypothalamus), and spinal cord, VR-3 and VR-5 may be involved in nociception (*e.g.*, chemical, mechanical, or thermal nociception) and thereby may modulate pain elicitation. Accordingly, the VR-3 and VR-5 molecules of the present invention act as targets for developing novel diagnostic targets and therapeutic agents to control pain and pain disorders.

As used herein, an "ion channel" includes a protein or polypeptide which is involved in receiving, conducting, and transmitting signals in an electrically excitable cell, *e.g.*, a neuronal or muscle cell. Ion channels include vanilloid receptors, calcium channels, potassium channels, and sodium channels. The VR-3 and VR-5 molecules of the present invention are highly expressed in kidney, indicating that these molecules may function as calcium channels. As used herein, a "calcium channel" includes a protein or polypeptide which is involved in receiving, conducting, and transmitting calcium ion-based signals in an electrically excitable cell. Calcium channels are calcium ion selective, and can determine membrane excitability (the ability of, for example, a neuronal cell to respond to a stimulus and to convert it into a sensory impulse). Calcium channels can also influence the resting potential of membranes, wave forms and frequencies of action potentials, and thresholds of excitation. Calcium channels are typically expressed in electrically excitable cells, *e.g.*, neuronal cells, and may form heteromultimeric structures (*e.g.*, composed of more than one type of subunit). Calcium

channels may also be found in non-excitabile cells (*e.g.*, adipose cells or liver cells), where they may play a role in, *e.g.*, signal transduction. Examples of calcium channels include the low-voltage-gated channels and the high-voltage-gated channels. Calcium channels are described in, for example, Davila *et al.* (1999) *Annals New York Academy of Sciences* 868:102-17 and McEnery, M.W. *et al.* (1998) *J. Bioenergetics and Biomembranes* 30(4): 409-418, the contents of which are incorporated herein by reference.

As used herein, a "vanilloid receptor" includes a non- selective cation channel that is structurally related to the TRP family of ion channels. Vanilloid receptors are also known as capsaicin receptors. Vanilloid receptors share several physical characteristics including an N-terminal cytoplasmic domain which contains three ankyrin repeats, six transmembrane domains, a pore-loop region located between transmembrane domains 5 and 6, and several kinase consensus sequences. Members of the vanilloid receptor (VR) family have been proposed to mediate the entry of extracellular calcium into cells, *e.g.*, in response to the depletion of intracellular calcium stores. VRs are typically expressed in nociceptive neurons among other cells types and are directly activated by harmful heat, extracellular protons, and vanilloid compounds. VRs may also be expressed in nonsensory tissues and may mediate inflammatory rather than acute thermal pain. Vanilloid receptors are described in, for example, Caterina, M.J. (1997) *Nature* 389:816-824 and Caterina, M.J. (2000) *Science* 288:306-313) the contents of which are incorporated herein by reference. As the VR-3 and VR-5 molecules of the present invention may modulate ion channel mediated activities (*e.g.*, calcium channel- and/or vanilloid receptor- mediated activities), they may be useful for developing novel diagnostic and therapeutic agents for ion channel associated disorders (*e.g.*, calcium channel and/or vanilloid receptor associated disorders).

As used herein, an "ion channel associated disorder" includes a disorder, disease or condition which is characterized by a misregulation of ion channel (*e.g.*, calcium channel) and/or vanilloid receptor) mediated activity. For example, a "calcium channel associated disorder" includes a disorder, disease or condition which is characterized by a misregulation of calcium channel mediated activity. A "vanilloid receptor associated disorder" includes a disorder, disease or condition which is characterized by a misregulation of vanilloid receptor mediated activity. Ion channel associated disorders,

e.g., calcium channel associated disorders and/or vanilloid receptor associated disorders, include CNS disorders, such as cognitive and neurodegenerative disorders, examples of which include, but are not limited to, Alzheimer's disease, dementias related to Alzheimer's disease (such as Pick's disease), Parkinson's and other Lewy diffuse body diseases, senile dementia, Huntington's disease, Gilles de la Tourette's syndrome, multiple sclerosis, amyotrophic lateral sclerosis, progressive supranuclear palsy, epilepsy, Jakob-Creutzfeldt disease, or AIDS related dementia; autonomic function disorders such as hypertension and sleep disorders, and neuropsychiatric disorders, such as depression, schizophrenia, schizoaffective disorder, Korsakoff's psychosis, mania, anxiety disorders, or phobic disorders; learning or memory disorders, *e.g.*, amnesia or age-related memory loss, attention deficit disorder, psychoactive substance use disorders, anxiety, phobias, panic disorder, as well as bipolar affective disorder, *e.g.*, severe bipolar affective (mood) disorder (BP-I), and bipolar affective neurological disorders, *e.g.*, migraine and obesity. Further CNS-related disorders include, for example, those listed in the American Psychiatric Association's Diagnostic and Statistical manual of Mental Disorders (DSM), the most current version of which is incorporated herein by reference in its entirety.

Ion channel associated disorders, *e.g.*, calcium channel disorders and/or vanilloid receptor associated disorders, also include pain disorders. As used herein, the term "pain disorder" includes a disorder affecting pain signaling mechanisms. Pain disorders include disorders characterized by aberrant (*e.g.*, excessive or amplified) pain. The VR-3 or VR-5 molecules may provide novel diagnostic targets and therapeutic agents to control pain in a variety of disorders, diseases, or conditions which are characterized by a deregulated, *e.g.*, upregulated or downregulated, pain response. For example, VR-3 or VR-5 molecules may provide novel diagnostic targets and therapeutic agents to control the exaggerated pain response elicited during various forms of tissue injury, *e.g.*, inflammation, infection, and ischemia, usually referred to as hyperalgesia (described in, for example, Fields, H.L. (1987) *Pain*, New York; McGraw-Hill). Further examples of pain and/or pain disorders include posttherapeutic neuralgia, diabetic neuropathy, postmastectomy pain syndrome, stump pain, reflex sympathetic dystrophy, trigeminal neuralgia, neuropathic pain, orofacial neuropathic pain, osteoarthritis, rheumatoid arthritis, fibromyalgia syndrome, tension myalgia, Guillian-Barre syndrome, Meralgia

paraesthesia, burning mouth syndrome, fibrositis, myofascial pain syndrome, idiopathic pain disorder, temporomandibular joint syndrome, atypical odontalgia, loin pain, haematuria syndrome, non-cardiac chest pain, low back pain, chronic nonspecific pain, pain associated with surgery, psychogenic pain, tooth pain, musculoskeletal pain disorder, chronic pelvic pain, nonorganic chronic headache, tension-type headache, cluster headache, migraine, complex regional pain syndrome, vaginismus, nerve trunk pain, somatoform pain disorder, cyclical mastalgia, chronic fatigue syndrome, multiple somatization syndrome, chronic pain disorder, somatization disorder, Syndrome X, facial pain, idiopathic pain disorder, posttraumatic rheumatic pain modulation disorder (fibrositis syndrome), hyperalgesia, and Tangier disease.

As used herein, the term "pain signaling mechanisms" includes the cellular mechanisms involved in the development and regulation of pain, *e.g.*, pain elicited by noxious chemical, mechanical, or thermal stimuli, in a subject, *e.g.*, a mammal such as a human. In mammals, the initial detection of noxious chemical, mechanical, or thermal stimuli, a process referred to as "nociception", occurs predominantly at the peripheral terminals of specialized, small diameter primary afferent neurons called polymodal nociceptors. These afferent neurons transmit the information to the central nervous system, evoking a perception of pain or discomfort and initiating appropriate protective reflexes. Vanilloid receptors, *e.g.*, the VR-3 and VR-5 molecules of the present invention, present on these afferent neurons, may be involved in detecting these noxious chemical, mechanical, or thermal stimuli and transducing this information into membrane depolarization events. Thus, the VR-3 and VR-5 molecules, by participating in pain signaling mechanisms, may modulate pain elicitation and provide novel diagnostic targets and therapeutic agents to control pain and pain disorders.

The VR-3 or VR-5 molecules of the present invention also play a role in calcium homeostasis. As used herein, the term "calcium homeostasis" includes cellular mechanisms involved in maintaining an equilibrium of intracellular or extracellular calcium concentration. Such mechanisms include the movement of calcium ions across cellular membranes (*e.g.*, intestine or kidney cellular membranes) in response to biological cues. The maintenance of calcium homeostasis is particularly important for an organism's nutritional needs. Important calcium transport processes are known to occur in the intestine and in the kidney. Thus, the VR-3 and VR-5 molecules, by

participating in calcium homeostasis mechanisms, can modulate calcium homeostasis mechanisms and provide novel diagnostic targets and therapeutic agents to control calcium homeostasis related disorders.

As used herein, the term "calcium homeostasis related disorders" includes disorders which are characterized by aberrant, *e.g.*, upregulated or downregulated, extracellular or intracellular calcium concentrations. Examples of such disorders include idiopathic hypercalciuria, sarcoidosis and other granulomatous disorders, primary hyperparathyroidism, diabetes, phosphorus depletion, osteoporosis, intrinsic bowel disease, hepatobiliary disease, renal disease, hyperthyroidism, and hypoparathyroidism, and CNS disorders, *e.g.*, Alzheimer's disease or Parkinson's disease.

The present invention is also based, at least in part, on the discovery that the VR-3 and VR-5 molecules are differentially expressed in tumors. VR-3 is differentially expressed in breast, colon, and prostate tumors as compared to normal breast, colon and prostate tissues. VR-5 is differentially expressed in lung, ovary, breast, and Wilms tumors, as compared to normal lung, ovary, breast, and kidney tissue. Accordingly, the VR-3 and VR-5 molecules of the present invention provide novel diagnostic targets and therapeutic agents to control cellular growth and/or proliferation disorders, *e.g.*, cancer.

As used herein, a "cellular growth and/or proliferation disorder" includes a disease or disorder that affects a cell growth or proliferation process. As used herein, a "cellular growth or proliferation process" is a process by which a cell increases in number, size or content, by which a cell develops a specialized set of characteristics which differ from that of other cells, or by which a cell moves closer to or further from a particular location or stimulus. A cellular growth or proliferation process includes the metabolic processes of the cell and cellular transcriptional activation mechanisms. A cellular growth or proliferation disorder may be characterized by aberrantly regulated cell growth, proliferation, differentiation, or migration. Cellular growth or proliferation disorders include tumorigenic disease or disorders. As used herein, a "tumorigenic disease or disorder" includes a disease or disorder characterized by aberrantly regulated cell growth, proliferation, differentiation, adhesion, or migration, resulting in the production of or tendency to produce tumors. As used herein, a "tumor" includes a normal benign or malignant mass of tissue. Examples of cellular growth or proliferation

disorders include, but are not limited to, cancer, *e.g.*, carcinoma, sarcoma, or leukemia, examples of which include, but are not limited to, colon, ovarian, lung, breast, endometrial, uterine, hepatic, gastrointestinal, prostate, and brain cancer; Wilms tumors; tumorigenesis and metastasis; skeletal dysplasia; and hematopoietic and/or
5 myeloproliferative disorders.

“Differential expression”, as used herein, includes both quantitative as well as qualitative differences in the temporal and/or tissue expression pattern of a gene. Thus, a differentially expressed gene may have its expression activated or inactivated in normal versus cellular growth or proliferation disease states. The degree to which
10 expression differs in normal versus cellular growth or proliferation disease states or control versus experimental states need only be large enough to be visualized via standard characterization techniques, *e.g.*, quantitative PCR, Northern analysis, or subtractive hybridization. The expression pattern of a differentially expressed gene may be used as part of a prognostic or diagnostic cellular growth or proliferation disorder
15 evaluation, or may be used in methods for identifying compounds useful for the treatment of cellular growth or proliferation disorder. In addition, a differentially expressed gene involved in tumorigenic disorders may represent a target gene such that modulation of the expression level of this gene or the activity of the gene product may act to ameliorate a cellular growth or proliferation disorder. Compounds that modulate
20 target gene expression or activity of the target gene product can be used in the treatment of cellular growth or proliferation disorders. Although the VR-3 and VR-5 genes described herein may be differentially expressed with respect to cellular growth or proliferation disorders, and/or their products may interact with gene products important to cellular growth or proliferation disorders, the genes may also be involved in
25 mechanisms important to additional tumor cell processes.

The term “family” when referring to the protein and nucleic acid molecules of the invention is intended to mean two or more proteins or nucleic acid molecules having a common structural domain or motif and having sufficient amino acid or nucleotide sequence homology as defined herein. Such family members can be naturally or non-
30 naturally occurring and can be from either the same or different species. For example, a family can contain a first protein of human origin, as well as other, distinct proteins of

human origin or alternatively, can contain homologues of non-human origin. Members of a family may also have common functional characteristics.

For example, the family of VR-3 or VR-5 proteins comprise at least one, and preferably five to six "transmembrane domains." As used herein, the term

5 "transmembrane domain" includes an amino acid sequence of about 15 amino acid residues in length which spans the plasma membrane. More preferably, a transmembrane domain includes about at least 10, 15, 20, 25, 30, 35, 40, 45 or more amino acid residues and spans the plasma membrane. Transmembrane domains are rich in hydrophobic residues, and typically have a helical structure. In a embodiment, at
10 least 50%, 60%, 70%, 80%, 90%, 95% or more of the amino acid residues of a transmembrane domain are hydrophobic, *e.g.*, leucines, isoleucines, tyrosines, or tryptophans. Transmembrane domains are described in, for example, Zagotta W.N. et al, (1996) *Annual Rev. Neurosci.* 19: 235-63, the contents of which are incorporated herein by reference. Amino acid residues 328-349, 386-402, 420-442, 456-482, 493-
15 512, and 553-577 of the human VR-3 polypeptide (SEQ ID NO:2) comprise transmembrane domains (Figure 6). Amino acid residues 466-490, 511-529, 551-568, 575-606, 617-636, and 693-717 of the human VR-5 polypeptide (SEQ ID NO:5) also comprise transmembrane domains (Figure 7).

In another embodiment, a VR-3 or VR-5 molecule of the present invention is
20 identified based on the presence of an "ankyrin repeat domain" in the protein or corresponding nucleic acid molecule. As used herein, the term "ankyrin repeat domain" includes a protein domain having an amino acid sequence of about 30-50 amino acid residues and having a bit score for the alignment of the sequence to the ankyrin repeat domain (HMM) of at least 6. Preferably, an ankyrin repeat domain includes at least
25 about 30-45, more preferably about 30-40 amino acid residues, or about 30-38 amino acids and has a bit score for the alignment of the sequence to the ankyrin repeat domain (HMM) of at least 2, 5-10, 10-20, 20-30, 30-40, 40-60 or greater. The ankyrin repeat domain HMM has been assigned the PFAM Accession PF00023 (<http://genome.wustl.edu/Pfam/.html>). Ankyrin repeats are involved in protein-protein
30 interactions and are described in, for example, Ketchum K.A., *et al.* (1996) *FEBS Letters* 378:19-26, the contents of which are incorporated herein by reference.

In another embodiment, a VR-3 or VR-5 molecule of the present invention is identified based on the presence of at least one "pore domain" between the fifth and sixth transmembrane domains. As used herein, the term "pore domain" includes an overall hydrophobic amino acid sequence which is located between two transmembrane domains of a calcium channel protein, preferably transmembrane domains 5 and 6, and which is believed to be a major determinant of ion selectivity and channel activity in calcium channels. Pore domains are described in, for example, Vannier *et al.* (1998) *J. Biol. Chem.* 273: 8675-8679 and Phillips, A. M. *et al.* (1992) *Neuron* 8, 631-642, the contents of which are incorporated herein by reference. VR-3 or VR-5 molecules having at least one pore domain are within the scope of the invention. Amino acid residues 523-544 of the human VR-3 polypeptide (SEQ ID NO:2) comprise a pore domain. Amino acid residues 666-683 of the human VR-5 polypeptide (SEQ ID NO:5) also comprise a pore domain.

In another embodiment, a VR-5 molecule of the present invention is identified based on the presence of an "ion transport protein domain." As used herein, the term "ion transport protein domain" includes a protein domain having an amino acid sequence of at least about 200-300, more preferably at least about 220-280 or at least about 235-260 amino acid residues and having a bit score for the alignment of the sequence to the ion transport protein domain (HMM) of at least about 1, 5, 10, 20, 30, 40, 50 or greater. The ion transport protein domain HMM has been assigned the PFAM Accession Number PF00520 (<http://genome.wustl.edu/Pfam/.html>). Proteins exhibiting this domain include sodium, potassium, and calcium ion channels.

To identify the presence of an ankyrin repeat domain or an ion transport protein domain in a VR-3 or VR-5 protein and make the determination that a protein of interest has a particular profile, the amino acid sequence of the protein is searched against a database of HMMs (*e.g.*, the Pfam database, release 2.1) using the default parameters (http://www.sanger.ac.uk/Software/Pfam/HMM_search). A description of the Pfam database can be found in Sonhammer *et al.* (1997) *Proteins* 28(3)405-420 and a detailed description of HMMs can be found, for example, in Gribskov *et al.* (1990) *Meth. Enzymol.* 183:146-159; Gribskov *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:4355-4358; Krogh *et al.* (1994) *J. Mol. Biol.* 235:1501-1531; and Stultz *et al.* (1993) *Protein Sci.* 2:305-314, the contents of which are incorporated herein by reference. A search

was performed against the HMM database resulting in the identification of three ankyrin repeat domains in the amino acid sequence of SEQ ID NO:2 (at about residues 78-108, 116-148, and 162-194). The search also identified the presence of three ankyrin repeat domains in SEQ ID NO:5 (at about residues 237-269, 284-319, and 369-400). The search further identified an ion transport protein domain in the amino acid sequence of SEQ ID NO:5 (at about residues 473-718). The results of this search are set forth in Figures 8 and 9.

Isolated VR-3 or VR-5 proteins of the present invention, have an amino acid sequence sufficiently identical to the amino acid sequence of SEQ ID NO:2 or 5, or are encoded by a nucleotide sequence sufficiently identical to SEQ ID NO:1, 3, 4 or 6. As used herein, the term "sufficiently identical" refers to a first amino acid or nucleotide sequence which contains a sufficient or minimum number of identical or equivalent (*e.g.*, an amino acid residue which has a similar side chain) amino acid residues or nucleotides to a second amino acid or nucleotide sequence such that the first and second amino acid or nucleotide sequences share common structural domains or motifs and/or a common functional activity. For example, amino acid or nucleotide sequences which share common structural domains have at least 30%, 40%, or 50% homology, preferably 60% homology, more preferably 70%-80%, and even more preferably 90-95% homology across the amino acid sequences of the domains and contain at least one and preferably two structural domains or motifs, are defined herein as sufficiently identical. Furthermore, amino acid or nucleotide sequences which share at least 30%, 40%, or 50%, preferably 60%, more preferably 70-80%, or 90-95% homology and share a common functional activity are defined herein as sufficiently identical.

As used interchangeably herein, a "VR-3 or VR-5 activity", "VR-3 activity", "VR-5 activity", "biological activity of VR-3", "biological activity of VR-5", "functional activity of VR-3", or "functional activity of VR-5", includes an activity exerted by a VR-3 or VR-5 protein, polypeptide or nucleic acid molecule on a VR-3- or VR-5-responsive cell or tissue, or on a VR-3 or VR-5 protein substrate, as determined *in vivo*, or *in vitro*, according to standard techniques. In one embodiment, a VR-3 or VR-5 activity is a direct activity, such as an association with a VR-3- or VR-5-target molecule. As used herein, a "target molecule" or "binding partner" is a molecule with which a VR-3 or VR-5 protein binds or interacts in nature, such that VR-3- or VR-5-mediated

function is achieved. A VR-3 or VR-5 target molecule can be a non-VR-3 or non-VR-5 molecule or a VR-3 or VR-5 protein or polypeptide of the present invention. In an exemplary embodiment, a VR-3 or VR-5 target molecule is a VR-3 or VR-5 ligand, *e.g.*, a vanilloid molecule or a vanilloid-containing compound such as capsaicin.

- 5 Alternatively, a VR-3 or VR-5 activity is an indirect activity, such as a cellular signaling activity mediated by interaction of the VR-3 or VR-5 protein with a VR-3 or VR-5 ligand, *e.g.*, a vanilloid or a vanilloid-containing compound such as capsaicin.

Preferably, a VR-3 or VR-5 activity is the ability to modulate the transmission of pain via, *e.g.*, pain signaling mechanisms. Also preferably, a VR-3 or VR-5 activity is the

- 10 ability to modulate the transport of calcium via, *e.g.*, calcium signaling mechanisms. In addition, a VR-3 or VR-5 activity also includes the modulation of cellular growth and/or proliferation and/or tumorigenesis.

Accordingly, another embodiment of the invention features isolated VR-3 or VR-5 polypeptides having a VR-3 or VR-5 activity. Preferred proteins are VR-3
15 proteins having at least one or more of the following domains: an ankyrin repeat domain, a pore domain, and/or a transmembrane domain, and, preferably, a VR-3 activity. Additional preferred VR-3 proteins have at least one ankyrin repeat domain and/or at least pore protein domain, and/or at least one transmembrane domain and are, preferably, encoded by a nucleic acid molecule having a nucleotide sequence which
20 hybridizes under stringent hybridization conditions to a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, 3, 4, or 6.

- Accordingly, a further embodiment of the invention features isolated VR-5 polypeptides having a VR-5 activity. Preferred proteins are VR-5 proteins having at least one or more of the following domains: an ankyrin repeat domain, a pore domain, a
25 transmembrane domain, and an ion transport protein domain, and, preferably, a VR-5 activity. Additional preferred proteins have at least one ankyrin repeat domain and/or at least one ion transport protein domain, and/or at least one pore domain, and/or at least one transmembrane domain, and are, preferably, encoded by a nucleic acid molecule having a nucleotide sequence which hybridizes under stringent hybridization conditions
30 to a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, 3, 4, or 6.

The nucleotide sequence of the isolated human VR-3 cDNA and the predicted amino acid sequence of the human VR-3 polypeptide are shown in Figure 1 and in SEQ ID NO:1 and SEQ ID NO:2, respectively.

The nucleotide sequence of the isolated human VR-5 cDNA and the predicted amino acid sequence of the human VR-5 polypeptide are shown in Figure 2 and in SEQ ID NO:4 and SEQ ID NO:5, respectively. A plasmid containing the nucleotide sequence encoding human VR-5 was deposited with the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110-2209, on June 8, 2000 and assigned Accession Number PTA-2013. This deposit will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. This deposits was made merely as a convenience for those of skill in the art and is not an admission that a deposit is required under 35 U.S.C. §112.

The human VR-3 gene, which is approximately 3026 nucleotides in length, encodes a protein having a molecular weight of approximately 79.8 kD and which is approximately 725 amino acid residues in length. The human VR-5 gene, which is approximately 3245 nucleotides in length, encodes a protein having a molecular weight of approximately 95.8 kD and which is approximately 871 amino acid residues in length.

Various aspects of the invention are described in further detail in the following subsections.

I. Isolated Nucleic Acid Molecules

One aspect of the invention pertains to isolated nucleic acid molecules that encode VR-3 or VR-5 proteins or biologically active portions thereof, as well as nucleic acid fragments sufficient for use as hybridization probes to identify VR-3- or VR-5-encoding nucleic acid molecules (*e.g.*, VR-3 mRNA, VR-5 mRNA) and fragments for use as PCR primers for the amplification or mutation of VR-3 or VR-5 nucleic acid molecules. As used herein, the term "nucleic acid molecule" is intended to include DNA molecules (*e.g.*, cDNA or genomic DNA) and RNA molecules (*e.g.*, mRNA) and analogs of the DNA or RNA generated using nucleotide analogs. The nucleic acid

molecule can be single-stranded or double-stranded, but preferably is double-stranded DNA.

The term "isolated nucleic acid molecule" includes nucleic acid molecules which are separated from other nucleic acid molecules which are present in the natural source of the nucleic acid. For example, with regards to genomic DNA, the term "isolated" includes nucleic acid molecules which are separated from the chromosome with which the genomic DNA is naturally associated. Preferably, an "isolated" nucleic acid is free of sequences which naturally flank the nucleic acid (*i.e.*, sequences located at the 5' and 3' ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, the isolated VR-3 or VR-5 nucleic acid molecule can contain less than about 5 kb, 4kb, 3kb, 2kb, 1 kb, 0.5 kb or 0.1 kb of nucleotide sequences which naturally flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid is derived. Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, can be substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized.

A nucleic acid molecule of the present invention, *e.g.*, a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:1, 3, 4, or 6, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number PTA-2013, or a portion thereof, can be isolated using standard molecular biology techniques and the sequence information provided herein. Using all or portion of the nucleic acid sequence of SEQ ID NO:1, 3, 4, or 6, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number PTA-2013, as a hybridization probe, VR-3 or VR-5 nucleic acid molecules can be isolated using standard hybridization and cloning techniques (*e.g.*, as described in Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual*. 2nd, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989).

Moreover, a nucleic acid molecule encompassing all or a portion of SEQ ID NO:1, 3, 4, or 6, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number PTA-2013 can be isolated by the polymerase chain reaction (PCR) using synthetic oligonucleotide primers designed based upon the

sequence of SEQ ID NO:1, 3, 4, or 6, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number PTA-2013.

A nucleic acid of the invention can be amplified using cDNA, mRNA or alternatively, genomic DNA, as a template and appropriate oligonucleotide primers according to standard PCR amplification techniques. The nucleic acid so amplified can be cloned into an appropriate vector and characterized by DNA sequence analysis. Furthermore, oligonucleotides corresponding to VR-3 or VR-5 nucleotide sequences can be prepared by standard synthetic techniques, *e.g.*, using an automated DNA synthesizer.

10 In a preferred embodiment, an isolated nucleic acid molecule of the invention comprises the nucleotide sequence shown in SEQ ID NO:1. The sequence of SEQ ID NO:1 corresponds to the human VR-3 cDNA. This cDNA comprises sequences encoding the human VR-3 protein (*i.e.*, "the coding region", from nucleotides 280-2452), as well as 5' untranslated sequences (nucleotides 1-279) and 3' untranslated
15 sequences (nucleotides 2453-3026). Alternatively, the nucleic acid molecule can comprise only the coding region of SEQ ID NO:1 (*e.g.*, nucleotides 1-2175, corresponding to SEQ ID NO:3).

In a further preferred embodiment, an isolated nucleic acid molecule of the invention comprises the nucleotide sequence shown in SEQ ID NO:4. The sequence of
20 SEQ ID NO:4 corresponds to the human VR-5 cDNA. This cDNA comprises sequences encoding the human VR-5 protein (*i.e.*, "the coding region", from nucleotides 84-2696), as well as 5' untranslated sequences (nucleotides 1-83) and 3' untranslated sequences (nucleotides 2697-3245). Alternatively, the nucleic acid molecule can comprise only the coding region of SEQ ID NO:1 (*e.g.*, nucleotides 1-2613, corresponding to SEQ ID
25 NO:6).

In another preferred embodiment, an isolated nucleic acid molecule of the invention comprises a nucleic acid molecule which is a complement of the nucleotide sequence shown in SEQ ID NO:1, 3, 4, or 6, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number PTA-2013, or a
30 portion of any of these nucleotide sequences. A nucleic acid molecule which is complementary to the nucleotide sequence shown in SEQ ID NO:1, 3, 4, or 6, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as

Accession Number PTA-2013, is one which is sufficiently complementary to the nucleotide sequence shown in SEQ ID NO:1, 3, 4, or 6, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number PTA-2013, such that it can hybridize to the nucleotide sequence shown in SEQ ID NO:1, 3, 4, or 6,
5 or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number PTA-2013, thereby forming a stable duplex.

In still another preferred embodiment, an isolated nucleic acid molecule of the present invention comprises a nucleotide sequence which is at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 83%, 85%, 86%, 90%, 91%, 92%, 95%, 96%, 97%, 98%,
10 99%, 99.1%, 99.5% or more identical to the entire length of the nucleotide sequence shown in SEQ ID NO:1, 3, 4, or 6, or the entire length of the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number PTA-2013, or a portion of any of these nucleotide sequences.

Moreover, the nucleic acid molecule of the invention can comprise only a
15 portion of the nucleic acid sequence of SEQ ID NO:1, 3, 4, or 6, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number PTA-2013, for example, a fragment which can be used as a probe or primer or a fragment encoding a portion of a VR-3 or VR-5 protein, *e.g.*, a biologically active portion of a VR-3 or VR-5 protein. The nucleotide sequence determined from the
20 cloning of the VR-3 or VR-5 gene allows for the generation of probes and primers designed for use in identifying and/or cloning other VR-3 or VR-5 family members, as well as VR-3 or VR-5 homologues from other species. The probe/primer typically comprises substantially purified oligonucleotide. The oligonucleotide typically comprises a region of nucleotide sequence that hybridizes under stringent conditions to
25 at least about 12 or 15, preferably about 20 or 25, more preferably about 30, 35, 40, 45, 50, 55, 60, 65, or 75 consecutive nucleotides of a sense sequence of SEQ ID NO:1, 3, 4, or 6, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number PTA-2013, of an anti-sense sequence of SEQ ID NO:1, 3, 4, or 6, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as
30 Accession Number PTA-2013, or of a naturally occurring allelic variant or mutant of SEQ ID NO:1, 3, 4, or 6, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number PTA-2013. In one embodiment, a nucleic

acid molecule of the present invention comprises a nucleotide sequence which is greater than 100-500, 500-1000, 1000-1500, 1500-2000, 2000-2500, 2500-3000, 3000-3200, or more nucleotides in length and hybridizes under stringent hybridization conditions to a nucleic acid molecule of SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number PTA-2013.

Probes based on the VR-3 or VR-5 nucleotide sequences can be used to detect transcripts or genomic sequences encoding the same or homologous proteins. In preferred embodiments, the probe further comprises a label group attached thereto, *e.g.*, the label group can be a radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor. Such probes can be used as a part of a diagnostic test kit for identifying cells or tissue which misexpress a VR-3 or VR-5 protein, such as by measuring a level of a VR-3 or VR-5 -encoding nucleic acid in a sample of cells from a subject, *e.g.*, detecting VR-3 or VR-5 mRNA levels or determining whether a genomic VR-3 or VR-5 gene has been mutated or deleted.

A nucleic acid fragment encoding a "biologically active portion of a VR-3 or VR-5 protein" can be prepared by isolating a portion of the nucleotide sequence of SEQ ID NO:1, 3, 4, or 6, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number PTA-2013, which encodes a polypeptide having a VR-3 or VR-5 biological activity (the biological activities of the VR-3 or VR-5 proteins are described herein), expressing the encoded portion of the VR-3 or VR-5 protein (*e.g.*, by recombinant expression *in vitro*) and assessing the activity of the encoded portion of the VR-3 or VR-5 protein.

The invention further encompasses nucleic acid molecules that differ from the nucleotide sequence shown in SEQ ID NO:1, 3, 4, or 6, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number PTA-2013, due to degeneracy of the genetic code and, thus, encode the same VR-3 or VR-5 proteins as those encoded by the nucleotide sequence shown in SEQ ID NO:1, 3, 4, or 6, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number PTA-2013. In another embodiment, an isolated nucleic acid molecule of the invention has a nucleotide sequence encoding a protein having an amino acid sequence shown in SEQ ID NO:2 or 5.

In addition to the VR-3 or VR-5 nucleotide sequences shown in SEQ ID NO:1, 3, 4, and 6, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number PTA-2013, it will be appreciated by those skilled in the art that DNA sequence polymorphisms that lead to changes in the amino acid sequences of the VR-3 or VR-5 proteins may exist within a population (*e.g.*, the human population). Such genetic polymorphism in the VR-3 or VR-5 genes may exist among individuals within a population due to natural allelic variation. As used herein, the terms "gene" and "recombinant gene" refer to nucleic acid molecules which include an open reading frame encoding a VR-3 or VR-5 protein, preferably a mammalian VR-3 or VR-5 protein, and can further include non-coding regulatory sequences, and introns.

Allelic variants of human VR-3 or VR-5 include both functional and non-functional VR-3 or VR-5 proteins. Functional allelic variants are naturally occurring amino acid sequence variants of the human VR-3 or VR-5 protein that maintain the ability to bind a VR-3 or VR-5 ligand or substrate and/or modulate pain signaling mechanisms, calcium homeostasis, cellular growth and/or proliferation, and/or tumorigenesis. Functional allelic variants will typically contain only conservative substitution of one or more amino acids of SEQ ID NO:2 or 5, or substitution, deletion or insertion of non-critical residues in non-critical regions of the protein.

Non-functional allelic variants are naturally occurring amino acid sequence variants of the human VR-3 or VR-5 proteins that do not have the ability to either bind a VR-3 or VR-5 ligand or substrate and/or modulate pain signaling mechanisms, calcium homeostasis mechanism, cellular growth and/or proliferation, and/or tumorigenesis. Non-functional allelic variants will typically contain a non-conservative substitution, a deletion, or insertion or premature truncation of the amino acid sequence of SEQ ID NO:2 or 5, or a substitution, insertion or deletion in critical residues or critical regions.

The present invention further provides non-human orthologues of the human VR-3 or VR-5 protein. Orthologues of the human VR-3 or VR-5 protein are proteins that are isolated from non-human organisms and possess the same VR-3 or VR-5 ligand binding and/or modulation of pain signaling mechanisms, modulation of calcium homeostasis mechanisms, modulation of cellular growth and/or proliferation, and/or modulation of tumorigenesis as the human VR-3 or VR-5 protein. Orthologues of the

human VR-3 or VR-5 protein can readily be identified as comprising an amino acid sequence that is substantially identical to SEQ ID NO:2 or 5.

Moreover, nucleic acid molecules encoding other VR-3 or VR-5 family members and, thus, which have a nucleotide sequence which differs from the VR-3 or VR-5 sequences of SEQ ID NO:1, 3, 4, or 6, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number PTA-2013 are intended to be within the scope of the invention. For example, another VR-3 or VR-5 cDNA can be identified based on the nucleotide sequence of human VR-3 or VR-5. Moreover, nucleic acid molecules encoding VR-3 or VR-5 proteins from different species, and which, thus, have a nucleotide sequence which differs from the VR-3 or VR-5 sequences of SEQ ID NO:1, 3, 4, or 6, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number PTA-2013 are intended to be within the scope of the invention. For example, a mouse VR-3 or VR-5 cDNA can be identified based on the nucleotide sequence of a human VR-3 or VR-5.

Nucleic acid molecules corresponding to natural allelic variants and homologues of the VR-3 or VR-5 cDNAs of the invention can be isolated based on their homology to the VR-3 or VR-5 nucleic acids disclosed herein using the cDNAs disclosed herein, or a portion thereof, as a hybridization probe according to standard hybridization techniques under stringent hybridization conditions. Nucleic acid molecules corresponding to natural allelic variants and homologues of the VR-3 or VR-5 cDNAs of the invention can further be isolated by mapping to the same chromosome or locus as the VR-3 or VR-5 gene.

Accordingly, in another embodiment, an isolated nucleic acid molecule of the invention is at least 15, 20, 25, 30 or more nucleotides in length and hybridizes under stringent conditions to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, 3, 4, or 6, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number PTA-2013. In other embodiment, the nucleic acid is at least 30, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 1000, 1200, 1400, 1600, 1800, 2000, 2200, 2242, 2400, 2500, 2600, 2700, 2800, 2900, 3000, 3100, 3200 or more nucleotides in length.

As used herein, the term "hybridizes under stringent conditions" is intended to describe conditions for hybridization and washing under which nucleotide sequences that are significantly identical or homologous to each other remain hybridized to each other. Preferably, the conditions are such that sequences at least about 70%, more preferably at least about 80%, even more preferably at least about 85% or 90% identical to each other remain hybridized to each other. Such stringent conditions are known to those skilled in the art and can be found in *Current Protocols in Molecular Biology*, Ausubel *et al.*, eds., John Wiley & Sons, Inc. (1995), sections 2, 4 and 6. Additional stringent conditions can be found in *Molecular Cloning: A Laboratory Manual*, Sambrook *et al.*, Cold Spring Harbor Press, Cold Spring Harbor, NY (1989), chapters 7, 9 and 11. A preferred, non-limiting example of stringent hybridization conditions includes hybridization in 4X sodium chloride/sodium citrate (SSC), at about 65-70°C (or hybridization in 4X SSC plus 50% formamide at about 42-50°C) followed by one or more washes in 1X SSC, at about 65-70°C. A preferred, non-limiting example of highly stringent hybridization conditions includes hybridization in 1X SSC, at about 65-70°C (or hybridization in 1X SSC plus 50% formamide at about 42-50°C) followed by one or more washes in 0.3X SSC, at about 65-70°C. A preferred, non-limiting example of reduced stringency hybridization conditions includes hybridization in 4X SSC, at about 50-60°C (or alternatively hybridization in 6X SSC plus 50% formamide at about 40-45°C) followed by one or more washes in 2X SSC, at about 50-60°C. Ranges intermediate to the above-recited values, *e.g.*, at 65-70°C or at 42-50°C are also intended to be encompassed by the present invention. SSPE (1xSSPE is 0.15M NaCl, 10mM NaH₂PO₄, and 1.25mM EDTA, pH 7.4) can be substituted for SSC (1xSSC is 0.15M NaCl and 15mM sodium citrate) in the hybridization and wash buffers; washes are performed for 15 minutes each after hybridization is complete. The hybridization temperature for hybrids anticipated to be less than 50 base pairs in length should be 5-10°C less than the melting temperature (T_m) of the hybrid, where T_m is determined according to the following equations. For hybrids less than 18 base pairs in length, $T_m(^{\circ}\text{C}) = 2(\# \text{ of A} + \text{T bases}) + 4(\# \text{ of G} + \text{C bases})$. For hybrids between 18 and 49 base pairs in length, $T_m(^{\circ}\text{C}) = 81.5 + 16.6(\log_{10}[\text{Na}^+]) + 0.41(\% \text{G} + \text{C}) - (600/\text{N})$, where N is the number of bases in the hybrid, and $[\text{Na}^+]$ is the concentration of sodium ions in the hybridization buffer ($[\text{Na}^+]$ for 1xSSC = 0.165 M). It will also be recognized by the